ORIGINAL ARTICLE

Contamination of environmental surfaces by genital human papillomaviruses

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Objective: To investigate contamination of environmental surfaces with human papillomaviruses (HPV) DNA in two genitourinary medicine (GUM) clinics and in an on-site staff leisure and fitness centre.
Methods: Samples were collected from the treatment rooms and patients' toilets of two GUM clinics situated at two hospital sites and from the toilets of the staff leisure and fitness centre on one of the sites. Samples were tested for the presence of HPV DNA by nested polymerase chain reaction (PCR), and HPV amplicons were typed by reverse line hybridisation using HPV type specific oligonucleotide probes complementary to 35 HPV types. All samples were also tested for β globin DNA by PCR in order to assess their quality.
Results: HPV DNA was found to be present at more than 50% of the sites sampled in one of the GUM clinics, but was absent in the second, and also from the staff leisure and fitness centre. All HPV DNA detected was found to be cell associated. The most commonly found HPV types were 6, 11, and 16, respectively. HPV infected cells were found to be localised mainly to surfaces used predominantly by medical staff.
Conclusions: This study has identified contamination of the environment of a GUM clinic. Possible sources for the contamination of the clinic toilets were from genital sites via hands to the environment. Within the treatment rooms the most likely route of HPV DNA contamination of the environment was via the doctor's gloved hands.

Genital human papillomaviruses (HPV) infections are spread predominantly through sexual intercourse, although other routes of transmission have been postulated,[1 2] and papillomaviruses may stay infectious within cells for up to 7 days, even after desiccation.[3] HPVs infect squamous epithelial cells, and desquamated cells are regularly shed from the skin and the mucous membranes. Virus particles contained within these cells may be transmitted between sexual partners and cause productive infection in the recipient. HPV infections are the most common sexually transmitted viral infections in the United Kingdom. To date there are more than 100 different HPV types distinguished on the basis of DNA sequence homology.[4] The replication cycle of HPV is heavily dependent on that of the host cell. This means that early stages of replication occur in basal cells, while the later stages of virus replication are only seen in differentiated epithelial cells. Clinically, HPVs have been subdivided into cutaneous and mucosal HPVs according to their site of infection. Infection with HPVs may result in asymptomatic infection or the development of clinically apparent lesions such as warts—that is, a localised proliferation of epithelial cells, on hands, feet, or within the genital tract. Patients with genital warts commonly present to genitourinary medicine (GUM) departments, and in 1999 there were 125 000 attendances in the United Kingdom for this reason (KC 60 returns). Genital warts are predominately caused by HPV types 6 and 11[5] although coinfection with other HPV types, in particular type 16,[6] is well documented.

There are a variety of therapeutic options for genital warts including cryotherapy and chemical treatments. Cryotherapy is a method used commonly for the removal of genital warts which may be applied by spraying liquid nitrogen onto the lesions, or by directly applying a probe cooled by liquid nitrogen. Chemical treatment with podophyllotoxin, which relies on local tissue destruction, may be self applied by the patient as a home treatment.

Although genital HPV types have been detected in non-genital epithelium and on non-epithelial surfaces,[7–9] the clinical significance of these findings is uncertain.

The objective of this study was to determine whether HPV DNA, and which types, were present on environmental surfaces in two GUM clinics and a hospital staff leisure and fitness centre, and to identify procedures associated with the risks of high levels of contamination.

MATERIALS AND METHODS

Environmental samples were collected from the treatment rooms and the male and female patients' toilets of GUM clinics A and B on six occasions and one occasion, respectively. The various sites sampled are listed in table 1. Environmental samples were also collected from male and female toilets at a hospital based staff leisure and fitness centre on one occasion. Samples were usually obtained during the working day, although the sampling at clinic A on the fifth and sixth occasion was performed at 8.30 am and 4.30 pm of the same day.

Samples were collected using cotton wool tipped swabs soaked in 0.1M phosphate buffered saline (PBS) pH 7.2. All environmental surfaces were swabbed firmly in order to pick up any cells that may have been present and gloves were changed between each sample. Samples were assigned a number on the arrival at the laboratory in order to mask the source of each individual sample during testing. Only after the testing of all samples was performed were laboratory numbers correlated with the sample source.

Serial dilutions of CaSki cells, containing 600 copies HPV-16 DNA per cell (starting concentration 4×10^6 cells/ml) and RNase-free water were included as positive and negative controls, respectively. Nucleic acids were extracted from all samples and controls using the guanidinium isothiocyanate/silica procedure.[10]

All samples were tested for the presence of β globin DNA using a real time PCR (LightCycler; Roche Diagnostics, UK),

Table 1 Detection of HPV in environmental samples collected from clinic A. (β Globin DNA could be detected in all HPV positive samples)

	Date of sampling; HPV type(s) detected				
	10 Oct 2000	31 Oct 2001	9 Nov 2000	9 Jan 2001	1 Feb 2001
Treatment room 1					
Bed control panel	None	None	None	11, 16, 31, 42	6, 16
Examination bed	None	None	6, 11	11, 16, 31, 42	6
Colposcope handle	None	None	None	Untyped	6, 16
Examination lamp	Untyped	None	None	16	6
Treatment room 2					
Bed control panel	None	None	6, 11, 16, 53, 66	6, 16	6, 16
Examination bed	None	6	6, 11, 16, 53	6, 16	Untyped
Colposcope handle	None	6,16,53	ND	6, 16	None
Examination lamp	None	6	18, 33, 53, CP8304	None	6, 16
Female toilet					
Toilet flush handle	None	6	None	None	None
Toilet seat	None	None	None	None	None
Door handle	None	6, 16	6, 16	No	None
Cold tap	None	None	None	None	None
Hot tap	None	None	None	None	None
Male toilet					
Door handle	None	None	None	None	6,16,62
Hot tap	None	None	None	6, 16, 72, CP8304	Untyped
Cold tap	None	None	None	None	6
Light switch	None	None	6,16	None	None
Cryoguns					
1	6, 42, 43, 45, Han 831	None	6, 11, 18, 58, 70	6, 11, 16, 51, 53	(pooled swabs) 6, 11, 18, 51, 59, 62
2	6, 11, 16, 53, 59	None	None	None	
3	6, 16, 43, 45, 59, 67	ND	ND	ND	
4	16, 43, 45, 67	ND	ND	ND	

ND=not done.

with the GH20/PC04 primers[11] before they were tested for HPV DNA, using a single tube nested real time PCR,[12] in order to determine the sample quality. The presence of β globin DNA was determined by melting the PCR product in order to measure its melting temperatures (Tm). The Tm values of the reaction products were compared to that of a positive control. A minimum of 15 copies of β globin DNA could be detected using this method.

HPV DNA was detected using the LightCycler, with the MY09/MY11[13] and GP5+/GP6+ primers[14] in a single tube nested PCR.[12] The presence of HPV DNA was determined by melting the PCR product in order to measure its Tm. A minimum of 10 HPV DNA input copies could be detected through the use of the single tube nested PCR.

Two microlitres, of HPV DNA positive, nested PCR amplicons were used to repeat the second round of the PCR with the GP5+ fluorophore labelled primer[15] and the GP6+ primer in order to generate products for HPV typing. These PCRs were carried out on a conventional block based thermal cycler (Perkin Elmer, GenAmp 2400) as described previously.[16]

Reverse line hybridisation was performed as described by Jordens *et al*.[15] Probes complementary to sequences of the L1 region of HPV types 2, 6, 11, 16, 18, 31, 33, 35, 39, 41, 42, 43, 45, 50, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 70, 72, Han 831, and CP8304 were used.

RESULTS

HPV DNA was detected in environmental samples collected from GUM clinic A on more than one occasion. HPV DNA was found mostly associated with surfaces of treatment areas and equipment used for the assessment and treatment of patients, although HPV DNA was also found in both the male and female patients' toilets (table 1). β Globin DNA was detected in all HPV DNA positive samples, and was not detected in any of the HPV negative samples collected from GUM clinic A. HPV DNA was not detected in any of the samples collected from GUM clinic B or from the staff leisure and fitness centre (table 2). β Globin DNA was not detected in any of the samples from the staff leisure and fitness centre and was found in only three samples from GUM clinic B (table 2).

Table 2 Sites sampled in clinic B and the staff leisure and fitness centre and the detection of β globin and HPV DNA

	1 Feb 2001	
	β Globin DNA detected (Yes/No)	HPV DNA detected (Yes/No)
Clinic B treatment room 1		
Examination bed	Yes	No
Colposcope handle	Yes	No
Light switch	No	No
Toilet light switch	Yes	No
Soap dispenser	No	No
Tap	No	No
Toilet seat	No	No
Toilet flush handle	No	No
Cryogun	No	No
Clinic B treatment rooms 2, 3, 4, and 5		
Examination bed	No	No
Colposcope handle	No	No
Light switch	No	No
Toilet light switch	No	No
Soap dispenser	No	No
Tap	No	No
Toilet seat	No	No
Toilet flush handle	No	No
Cyoguns	No	No
Hospital based staff leisure and fitness centre male toilets and female toilets each:		
Toilet seat 2×	No	No
Toilet flush handle 2×	No	No
Taps 2×	No	No
Light switch	No	No
Door handle	No	No

Table 3 Accumulation of HPV DNA in GUM clinic A during one day. (β Globin DNA could be detected in all HPV positive samples)

	HPV type(s) detected	
	8.30 am	4.30 pm
Treatment room 1		
Bed control panel	None	6, 16
Examination bed	None	6
Colposcope handle	None	6, 16
Examination lamp	None	6
Treatment room 2		
Bed control panel	None	6, 16
Examination bed	None	Untyped
Colposcope handle	None	None
Examination lamp	None	6, 16
Female toilet		
Toilet flush handle	ND	6, 16, 35, 59
Toilet seat	Untyped	None
Door handle	16	None
Cold tap	None	None
Hot tap	None	None
Male toilet		
Door handle	Untyped	6, 16, 62
Hot tap	None	Untyped
Cold tap	None	6
Light switch	None	None
Cryoguns		
Pooled swabs	6, 11	6, 11, 18, 51, 59, 62

ND=not done.

Table 3 shows the accumulation of HPV types throughout GUM clinic A during a single day. At 8.30 am HPV DNA was detected only in samples from the female and male toilets, and on the surface of the cryotherapy guns. In samples collected at 4.30 pm, HPV DNA was detected throughout the clinic, toilets, and on the equipment used for the assessment and treatment of patients (Table 3).

A total of 19 HPV types, all associated with genital HPV infection, were detected on environmental surfaces. HPV types 6, 16, and 11 were the most commonly found types, accounting for 28.6%, 24.2%, and 9.8%, respectively, of all HPV types detected in the environment. These correspond to the distribution and prevalence of HPV types identified from genital swabs of GUM clinic patients on different occasions (unpublished data).

DISCUSSION

The aim of this study was to investigate the possibility of environmental contamination with genital HPV types in "high risk" and "low risk" environments.

HPV infected cells were shown to be present on many of the surfaces within the treatment rooms in GUM clinic A. The spectrum of HPV types detected was similar to that found in the attendees of this GUM clinic (unpublished data).

The failure to type four of the HPV amplicons may have been due to the absence of a specific probe, complementary to sequences in the PCR amplicon. The probes used were specific for fewer than 50% of known HPV types, although they were chosen to match the spectrum of mainly genital HPV types detected in the population of the United Kingdom.

All identified HPV types detected in the treatment rooms were mucosal HPV types, ruling out the possibility of contamination from cutaneous wart virus. HPV DNA detected on the bed control panel, light switch, and the colposcope handle were much more likely to indicate contamination from the doctor's gloved hands after he/she had examined the patient. Contamination of the examination bed and of the patients' toilets is more likely to have arisen from the patients themselves, as these are areas with which medical and nursing staff have little or no contact. The toilets sampled were used exclusively by patients, and all HPV types detected were of mucosal origin indicating transfer from genital sites to the environment via hands or by direct contact. HPV DNA was detected at a higher frequency in the male toilets then in the female toilets of GUM clinic A, and only two HPV types were detected in the female toilets compared to five HPV types in the male toilets, again suggesting the transfer of HPV DNA was from the genitalia via the hands onto the environmental surfaces. Hand carriage of genital HPV DNA has been shown in a previous study, where patients with genital HPV infections were found to carry the same HPV type on their hands.[9]

The cleaning regimes employed in the clinics and the space available for treating patients were factors associated with the presence or absence of contamination. GUM clinic A had no formal cleaning procedures in place, whereas GUM clinic B had established very rigorous and strict cleaning procedures. The patients' toilets of clinic A were cleaned once a day by the contract cleaning staff. Treatment rooms, as well as equipment, were only cleaned at the end of each week, with the exception of the examination bed, which was cleaned at the end of each day. GUM clinic A houses two small treatment rooms and one male and female toilet adjacent to the waiting area. GUM clinic B was very spacious, with six new treatment rooms, each with their own adjacent patient toilet.

In GUM clinic A equipment used, such as cryotherapy guns, was frequently moved between treatment rooms and was never cleaned. In GUM clinic B, individual cryotherapy spray guns were used less frequently, owing to greater use of self treatment, and were allocated to each room and after use were wiped down with Azowipe (70% isopropyl alcohol BP, Vernon-Carus Ltd, UK). Interestingly, eight out of the 14 HPV types detected on the cryoguns of clinic A were HPV types which were never detected on any environmental surfaces within the clinic. All these eight HPV types (43, 45, 51, 58, 59, 67, 70, Han 831) are associated with cervical abnormalities or neoplasia rather then clinically obvious lesions.

The number of clinic attendees in GUM clinic A was higher than that of clinic B, resulting in the throughput for each individual room being higher for GUM clinic A than GUM clinic B. Out of a total of approximately 100 patients attending GUM clinic A per day up to 20 had genital warts. However, in GUM clinic B a total of 19 patients, of which only three patients had warts and four patients attended for colposcopy, were seen on the day of sampling. On the day before sampling a total of 25 patients, of whom three had genital warts, were seen in GUM clinic B.

Samples obtained before and after working hours in GUM clinic A showed an absence of HPV and β globin DNA before patients attending. This suggests degradation of cellular and HPV DNA which had contaminated surfaces on the previous working day.

The detection of HPV DNA does not necessarily indicate the presence of viable virus. The lack of in vitro culture procedures for HPV at present does not allow the viability of the virus to be determined. The correlation between the detection of HPV DNA and β globin DNA would suggest that only cell associated viral DNA was recovered. Bovine papillomavirus type 1 has been shown to survive desiccation in cell extracts for up to 7 days.[3]

The quantity of virus present on environmental surfaces was not determined. However, the single tube real time nested PCR enables the detection of a minimum of 10 input copies of HPV DNA; therefore, even a very small number of HPV DNA present could have been recovered from the sites sampled in clinic A. It is possible that even if viable virus was present this may be insufficient to infect an epithelial site by hand transfer. Given that the possibility of transmission via environmental surfaces is neither confirmed nor disproved, precautions should be taken to prevent the transfer of infected cells via human "vectors." Regular cleaning of the clinic environment

and equipment would reduce the potential for infected cells to contaminate environmental surfaces. It is likely that the more rigorous cleaning regime of GUM clinic B, together with fewer patient attendances was the reason for the absence of detectable β globin and HPV DNA. GUM clinic A has implemented a more rigorous cleaning policy since we undertook this study, and we hope to re-examine the issue of environmental viral contamination in the near future.

CONTRIBUTORS

The principal author, SS, with the co-author, PS, collected the samples, and performed the PCR and the reverse hybridisation on the environmental samples; CS supervised the sample collection in GUM clinic A and was co-author; SE supervised the sample collection in GUM clinic B and was co-author; JG supervised the project and was senior author.

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